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Supplemental Information

Independent, Reciprocal Neuromodulatory Control of Sweet and Bitter Taste Sensitivity during Starvation in *Drosophila*

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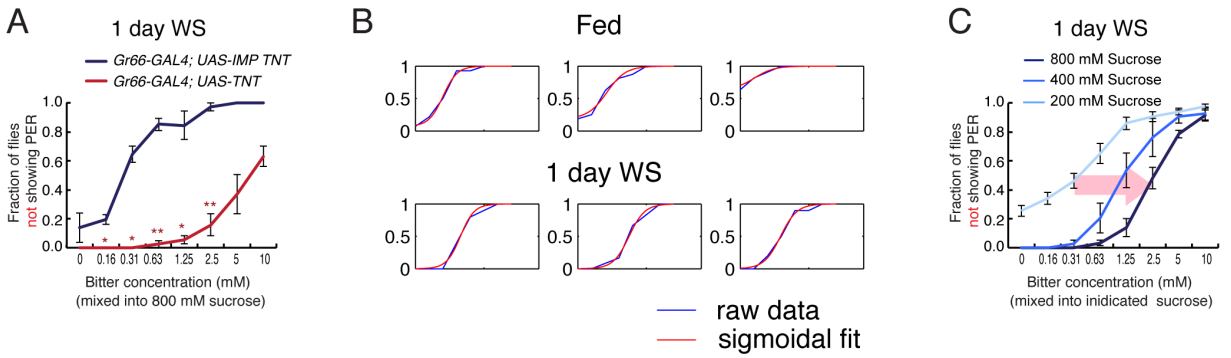


Figure S1

Figure S1. Modulation of Bitter Sensitivity During Starvation

(A) Gr66 GRNs are necessary for the bitter-dependent suppression of PER. Fraction of flies not showing PER to different concentrations of lobeline mixed into 800mM sucrose are plotted. (B) Multiple representative examples of sigmoidal fitting (red curves) of fraction of flies not showing PER (raw data in blue curves). See Supplemental Experimental Procedures for sigmoidal fitting. (C) Fraction of flies not showing PER in response to bitter mixed into different concentrations of sucrose solution. Note that high concentration of sucrose masks the effect of bitter to suppress PER. (A, C) n>4 for all experimental groups.

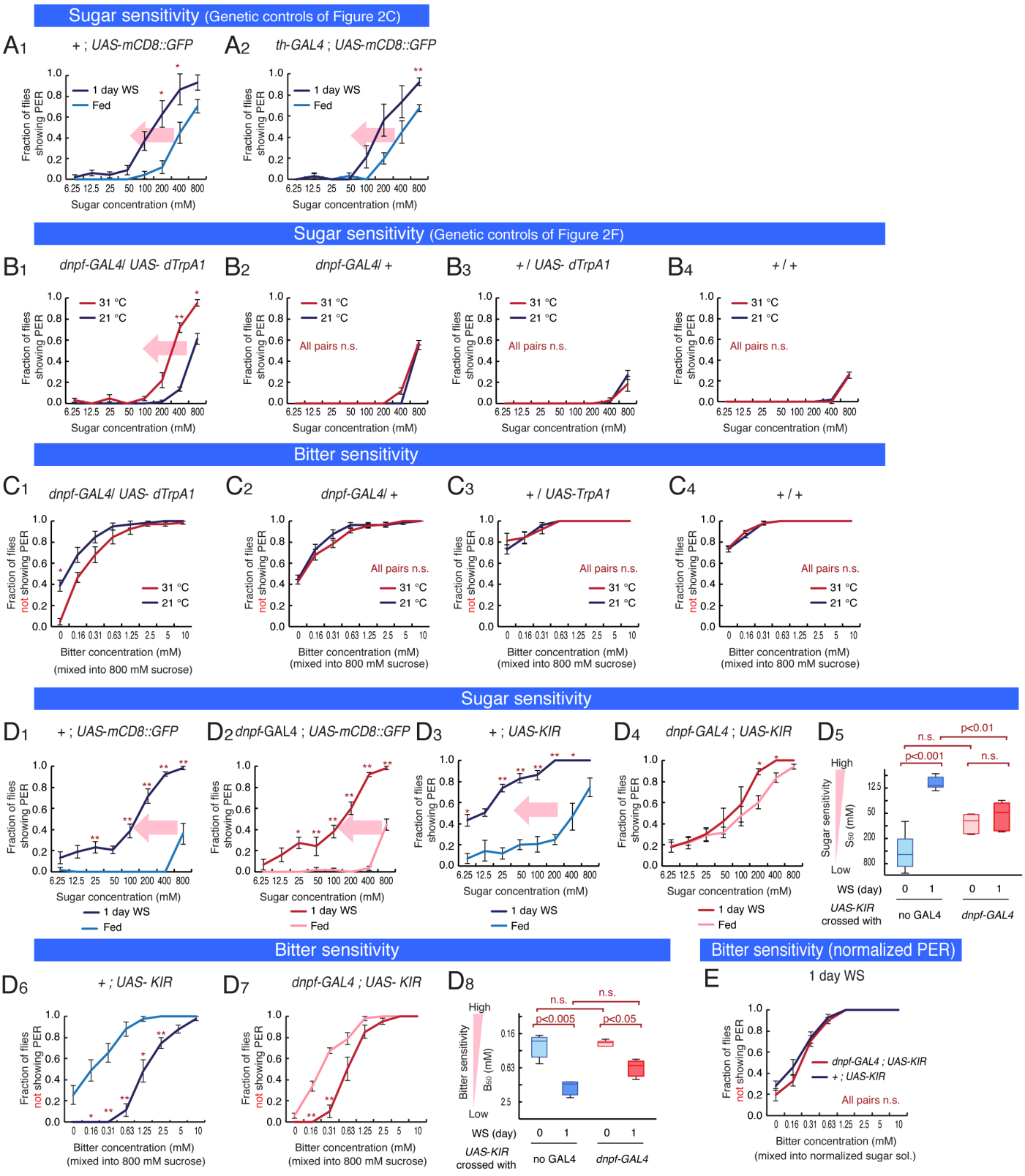

Sugar sensitivity (Genetic controls of Figure 2C)
A1
+ ; UAS-mCD8::GFP
A2
th-GAL4 ; UAS-mCD8::GFP
Fraction of flies showing PER
1 day WS
Fed
Sugar concentration (mM)
Sugar sensitivity (Genetic controls of Figure 2F)
B1
dnpf-GAL4/ UAS- dTrpA1
B2
dnpf-GAL4/ +
B3
+ / UAS- dTrpA1
B4
+ / +
31 °C
21 °C
All pairs n.s.
Bitter sensitivity
C1
dnpf-GAL4/ UAS- dTrpA1
C2
dnpf-GAL4/ +
C3
+ / UAS-TrpA1
C4
+ / +
Fraction of flies not showing PER
Bitter concentration (mM)
(mixed into 800 mM sucrose)
Sugar sensitivity
D1
+ ; UAS-mCD8::GFP
D2
dnpf-GAL4 ; UAS-mCD8::GFP
D3
+ ; UAS-KIR
D4
dnpf-GAL4 ; UAS-KIR
D5
Sugar sensitivity
High
Low
S50 (mM)
n.s.
p<0.01
p<0.001
WS (day)
UAS-KIR crossed with
no GAL4
dnpf-GAL4
Bitter sensitivity
D6
+ ; UAS- KIR
D7
dnpf-GAL4 ; UAS- KIR
D8
p<0.005
p<0.05
Bitter sensitivity (normalized PER)
E
1 day WS
dnpf-GAL4 ; UAS-KIR
+ ; UAS-KIR
Bitter concentration (mM)
(mixed into normalized sugar sol.)
F1
dnpf-GAL4;
UAS-mCD8::GFP
F2
α Tyrosine Hydroxylase
F3
merge

Figure S2

Figure S2. Neuronal Pathway Regulating Sugar Sensitivity Does Not Affect Bitter Sensitivity

(A) Genetic control of Figure 2C. Note that these genetic manipulations do no affect sugar sensitivity. (B-C) Sugar (B) and bitter (C) sensitivity of flies with thermogenetic activation of NPF neurons. Genotypes: w-; *dnpf-GAL4* (II) flies were crossed with w-; *UAS-dTrpA1* (II); *UAS-dTrpA1* (III) (B_1 and C_1) or w- flies in the same genetic background (B_2 and C_2); w- flies were crossed with w-; *UAS-dTrpA1* (II); *UAS-dTrpA1* (III) (B_3 and C_3) or w- flies in the same genetic background (B_4 and C_4). B_1 is copied from figure $2F_1$ for purposes of comparison. In (C_1), note that there is a statistically significant difference only when bitter is not mixed into sucrose solution (0 mM). Therefore, no difference in bitter sensitivity was observed. (D) Sugar and bitter sensitivity of flies with genetic silencing of dNPF neurons. *UAS-mCD8::GFP* was crossed with either w-; d*npf-GAL4* (II) flies (D_1) or w- flies in the same genetic background (D_2). *UAS-KIR2.1* was crossed with either w-; d*npf-GAL4* (II) flies (D_4 and D_7) or w- flies in the same genetic background (D_3 and D_6). (E) Comparison of bitter sensitivity of 1-day WS *dnpf-GAL4; UAS-KIR* flies and *+; UAS-KIR* flies using the sugar-normalized PER assay (200 mM and 100 mM sucrose solution were used, respectively). No difference in bitter sensitivity was observed between two genotypes. (F) Representative confocal projections of whole mount brains from *dnpf-GAL4*; *UAS-mCD8::GFP* flies (GFP in green: $F_{1,3}$) immunostained with anti-Tyrosine hydroxylase antibody (magenta: $F_{2\text{-}3}$), which labels DA neurons. (A-E) n>4 for all experimental groups.

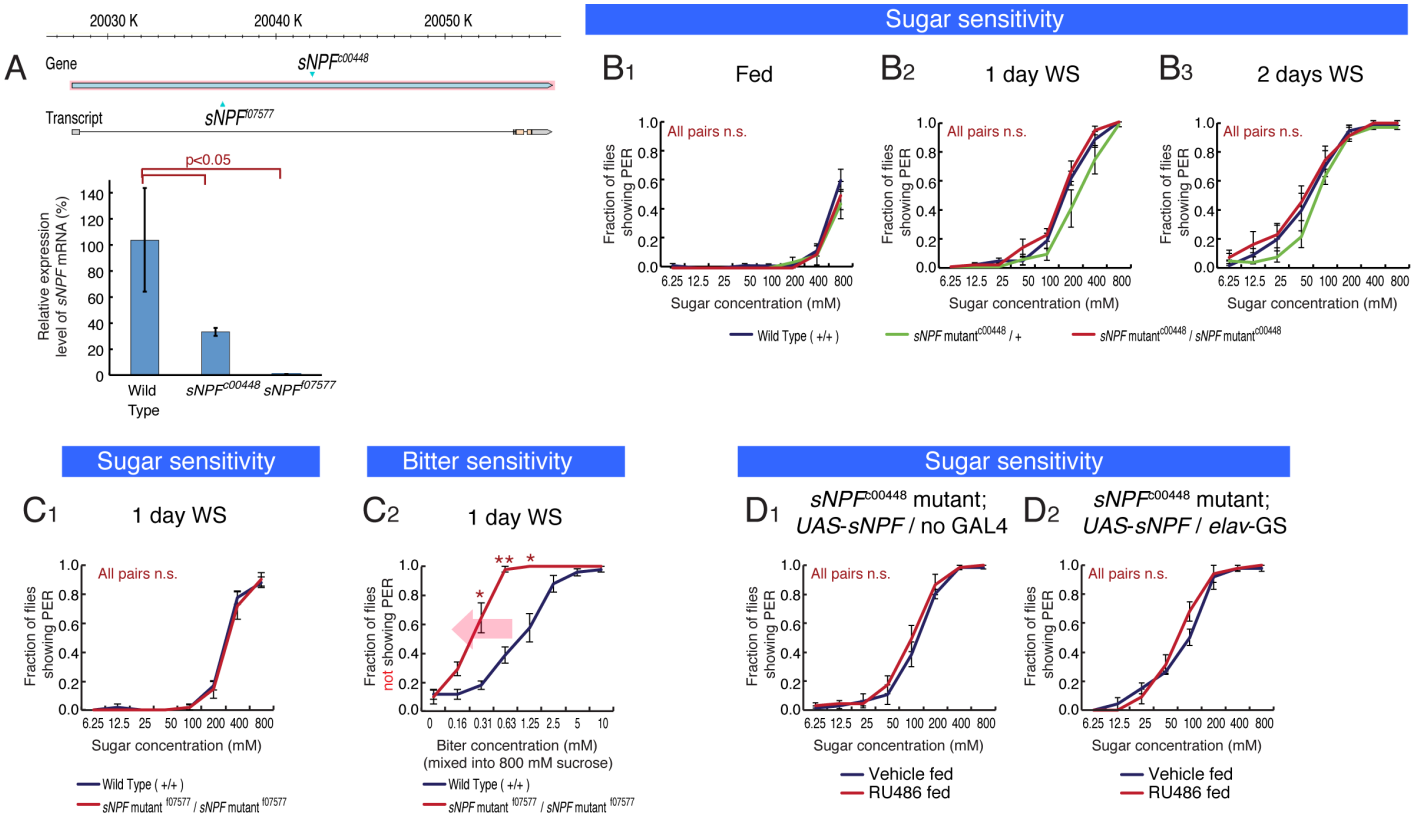
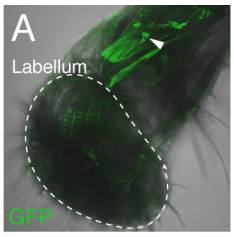
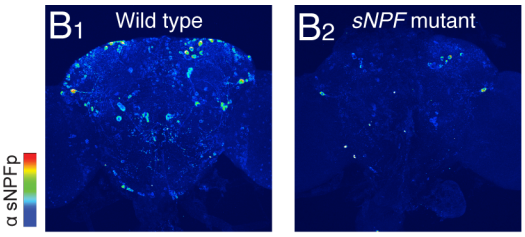


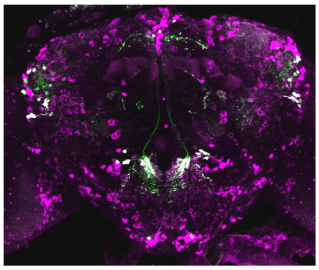
Figure S3

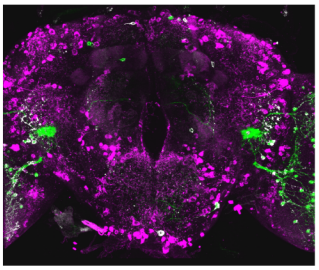
Figure S3. Genetic Manipulations of sNPF Do Not Affect Sugar Sensitivity

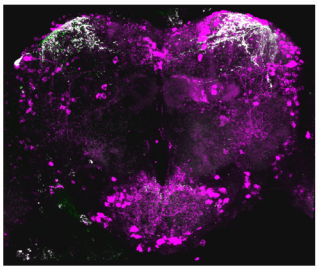
(A) Insertion of two piggyBac transposons in *sNPF* gene locus (top) and relative *sNPF* mRNA expression level of these strains compared to wild type flies in the same genetic background measured by qPCR (bottom). One-way ANOVA followed by post hoc t-test with Bonferroni correction (n=3). The top panel is modified from flybase (http://flybase.org/). (B) Sugar sensitivity of wild type and *sNPF* mutant flies. Data is acquired from the same flies that were used in Figure $3A_{1\text{-}3}$. S_{50} is summarized in Figure $3B_1$. (C) Sugar and bitter sensitivity of *sNPF* ^{f07577} flies compared with wild type flies in the same genetic background. (D) Sugar sensitivity of flies with pan-neuronal rescue of sNPF. Data is acquired from the same flies that were used in Figure $3D_{1\text{-}2}$. S_{50} is summarized in Figure $3E_1$. n>5 for all experimental groups.

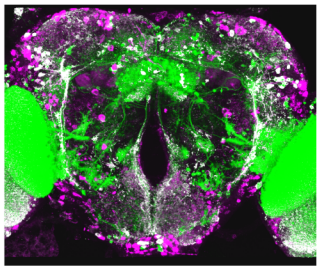

A
Labellum
GFP

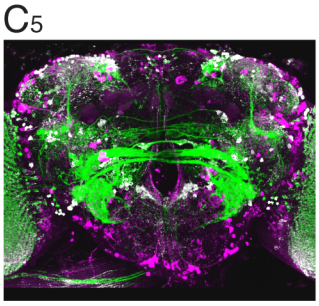

B1 Wild type
B2 sNPF mutant
α sNPFp

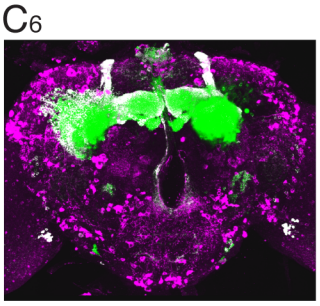

C1
α sNPF
GFP (GMR20E08-GAL4; UAS-mCD8::GFP)

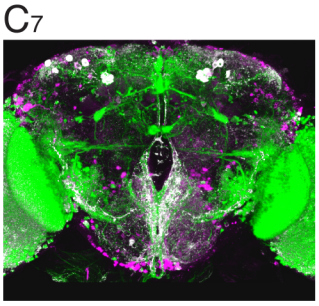

C2
α sNPF
GFP (GMR20B12-GAL4; UAS-mCD8::GFP)

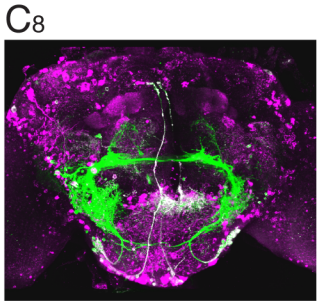

C3
α sNPF
GFP (GMR19H06-GAL4; UAS-mCD8::GFP)

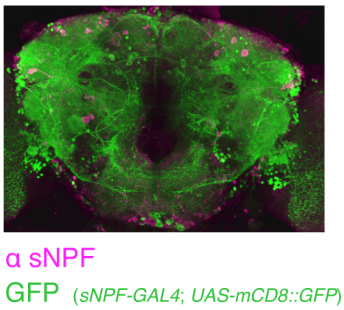

C4
α sNPF
GFP (GMR21A02-GAL4; UAS-mCD8::GFP)


C5
α sNPF
GFP (GMR21A12-GAL4; UAS-mCD8::GFP)


C6
α sNPF
GFP (GMR21C05-GAL4; UAS-mCD8::GFP)


C7
α sNPF
GFP (GMR21B01-GAL4; UAS-mCD8::GFP)


C8
α sNPF
GFP (GMR19H12-GAL4; UAS-mCD8::GFP)


D
α sNPF
GFP (sNPF-GAL4; UAS-mCD8::GFP)

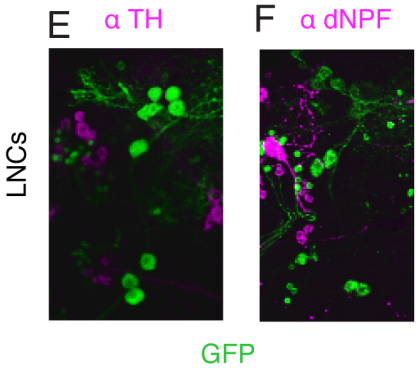

E α TH
F α dNPF
LNCs
GFP
(GMR21B10-GAL4; UAS-mCD8::GFP)

Figure S4

Figure S4. Expression Patterns of sNPF-promoter GAL4s

(A) Representative confocal projections of the proboscis from *GMR21B10-GAL4*; *UAS-mCD8::GFP* flies (Green: GFP; gray: DIC image of proboscis). Note that there is no GFP positive cell in labellum where GRNs exist. There are two cells in other parts of labellum (white arrow head). (B) Representative confocal projections of whole mount brains from wild type (B_1) or *sNPF*^{c00448} (B_2) flies immunostained with anti-sNPF antibody. Scale bar to the left represents relative intensity of immunostaining in pseudocolor. (C) Representative confocal projections of whole mount brains from sNPF promoter GAL4 lines crossed with *UAS-mCD8*::*GFP*. Green: GFP; Magenta: anti-sNPF signal. Overlap of GFP and anti-sNPF signal is emphasized by overlaying white color. (D) Representative confocal projections of whole mount brains from s*npf-GAL4*; *UAS-mCD8::GFP* flies (GFP: green) immunostained with anti-sNPF antibody (magenta). Note that huge populations of neurons in the brain are labeled by this GAL4. Some of them are sNPF positive. 3-4 LCNs are labeled by this GAL4 line. (E-F) Representative confocal projections of whole mount brains from *GMR21B10-GAL4*; *UAS-mCD8::GFP* flies immunostained with anti-Tyrosine hydroxylase (TH) (E) or anti-dNPF (F) antibodies. Note that LNCs are not TH or dNPF positive.

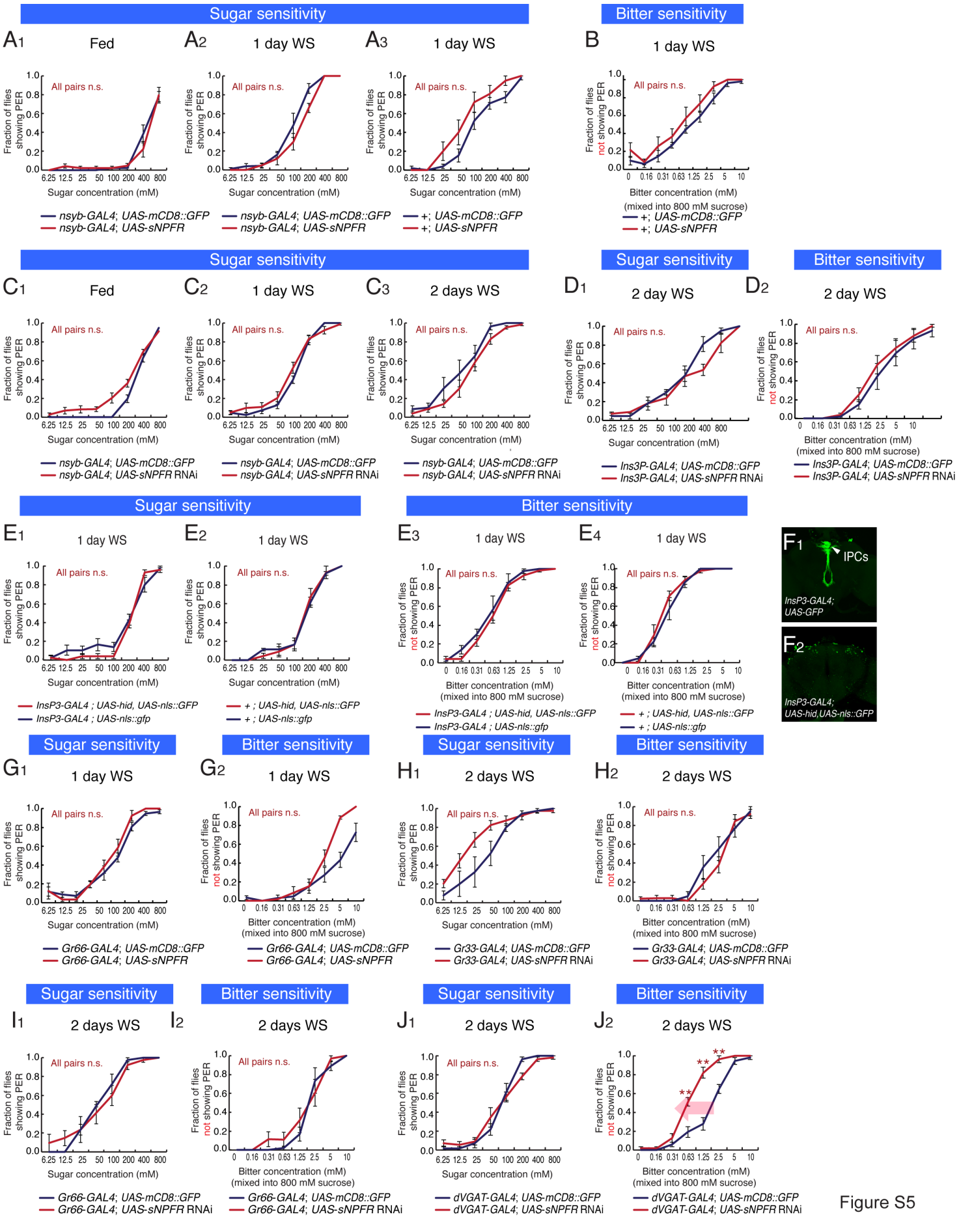
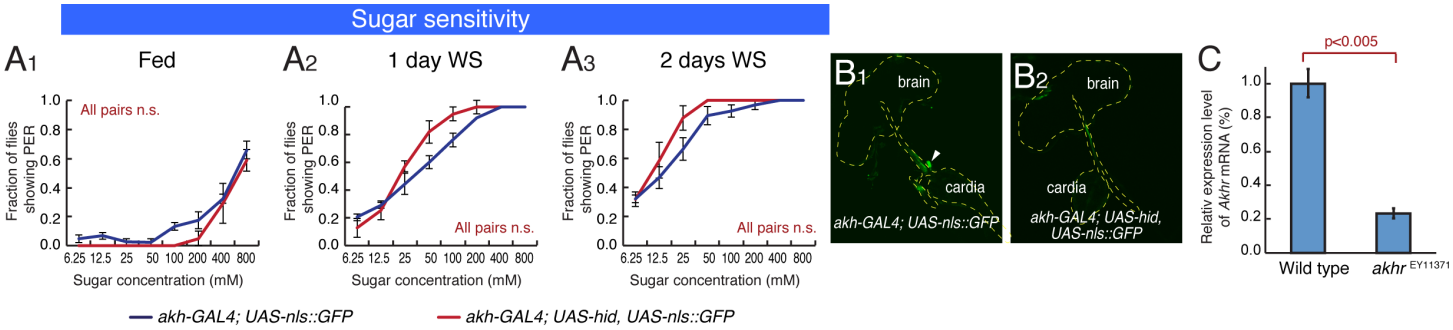
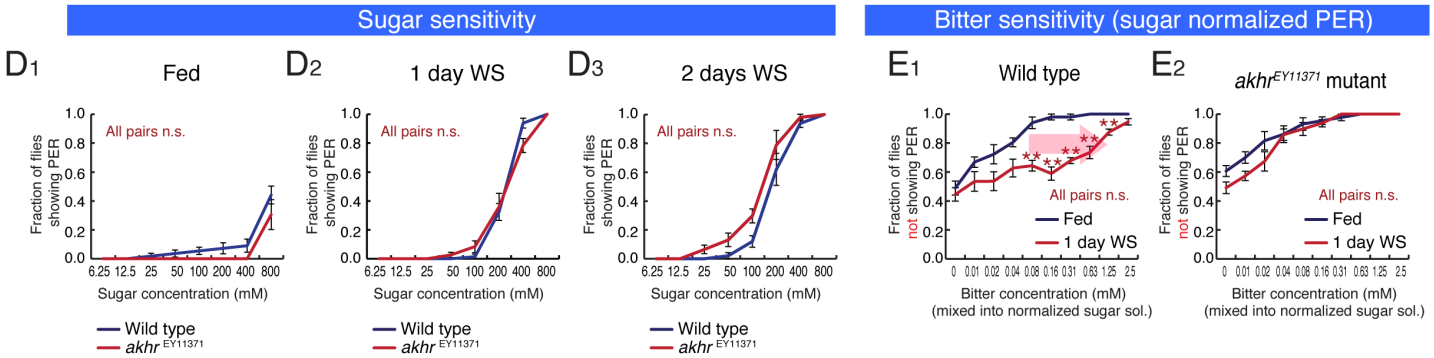

Sugar sensitivity
Bitter sensitivity
A1 Fed
A2 1 day WS
A3 1 day WS
B 1 day WS
C1 Fed
C2 1 day WS
C3 2 days WS
D1 2 day WS
D2 2 day WS
E1 1 day WS
E2 1 day WS
E3 1 day WS
E4 1 day WS
F1 IPCs
InsP3-GAL4; UAS-GFP
F2
InsP3-GAL4; UAS-hid,UAS-nls::GFP
G1 1 day WS
G2 1 day WS
H1 2 days WS
H2 2 days WS
I1 2 days WS
I2 2 days WS
J1 2 days WS
J2 2 days WS
All pairs n.s.
Fraction of flies showing PER
Fraction of flies not showing PER
Sugar concentration (mM)
Bitter concentration (mM)
(mixed into 800 mM sucrose)
nsyb-GAL4; UAS-mCD8::GFP
nsyb-GAL4; UAS-sNPFR
+; UAS-mCD8::GFP
+; UAS-sNPFR
nsyb-GAL4; UAS-mCD8::GFP
nsyb-GAL4; UAS-sNPFR RNAi
Ins3P-GAL4; UAS-mCD8::GFP
Ins3P-GAL4; UAS-sNPFR RNAi
InsP3-GAL4 ; UAS-hid, UAS-nls::GFP
InsP3-GAL4 ; UAS-nls::gfp
+ ; UAS-hid, UAS-nls::GFP
+ ; UAS-nls::gfp
Gr66-GAL4; UAS-mCD8::GFP
Gr66-GAL4; UAS-sNPFR
Gr33-GAL4; UAS-mCD8::GFP
Gr33-GAL4; UAS-sNPFR RNAi
dVGAT-GAL4; UAS-mCD8::GFP
dVGAT-GAL4; UAS-sNPFR RNAi

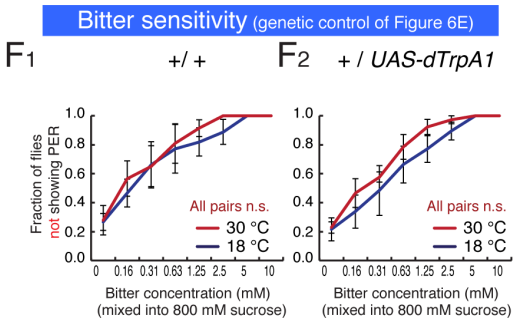
Figure S5

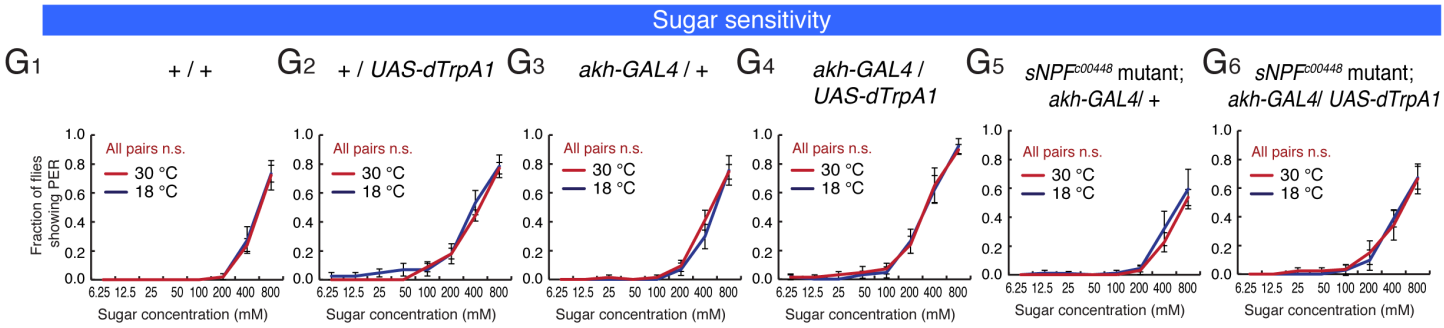
Figure S5. Genetic Manipulations of sNPFR Do Not Affect Sugar Sensitivity

(A-B) Sugar and bitter sensitivity of flies with genetic over-expression of sNPFR. Data is acquired from the same flies that were used in Figure $5A_{1-2}$. S_{50} is summarized in Figure $5B_1$. (C) Sugar sensitivity of flies with genetic knock-down of sNPFR. Data is acquired from the same flies that were used in Figure $5C_{1-3}$. S_{50} is summarized in Figure $5D_1$. (D) Sugar and bitter sensitivity of flies with genetic knock-down of sNPFR in IPCs by using *InsP3-GAL4*, GAL4 line specifically labeling IPCs, crossed with *UAS-sNPFR RNAi* or *UAS-mCD8::GFP* in the same genetic background. Note that no change was observed in gustatory sensitivity. (E-F) Sugar and bitter sensitivity of flies with genetic cellular ablation of IPCs by using *InsP3-GAL4* (or control wild type flies in the same genetic background) crossed with *UAS-hid* or *UAS-nls::GFP* in the same genetic background. Ablation of IPCs were confirmed as a loss of GFP signal (F). (G-I) Sugar and bitter sensitivity of flies with over-expression or genetic knock-down of sNPFR in bitter-sensing GRNs. Both *Gr66-GAL4* and *Gr33-GAL4* drivers were tested for RNAi also combined with *UAS-Dicer2* (*UAS-Dicer2*; *Gr66-GAL4* or *UAS-Dicer2*; *Gr33-GAL4* crossed with *UAS-sNPFR RNAi* or *UAS-mCD8::GFP* in the same genetic background). Similar results (no effect on gustatory sensitivity) were observed for 1-day WS flies (data not shown). (J) Sugar and bitter sensitivity of flies with genetic knock-down of sNPFR in GABA positive neurons. *dVGAT-GAL4* line is combined with *UAS-sNPFR RNAi* or *UAS-mCD8::GFP* in the same genetic background. *UAS-Dicer2* was not used for this experiment. n>5 for all experimental groups.


Sugar sensitivity
A1
Fed
A2
1 day WS
A3
2 days WS
All pairs n.s.
Fraction of flies showing PER
Sugar concentration (mM)
6.25 12.5 25 50 100 200 400 800
B1
brain
cardia
akh-GAL4; UAS-nls::GFP
B2
brain
cardia
akh-GAL4; UAS-hid, UAS-nls::GFP
C
p<0.005
Relativ expression level of Akhr mRNA (%)
Wild type
akhr EY11371
akh-GAL4; UAS-nls::GFP
akh-GAL4; UAS-hid, UAS-nls::GFP


Sugar sensitivity
D1
Fed
D2
1 day WS
D3
2 days WS
All pairs n.s.
Fraction of flies showing PER
Sugar concentration (mM)
6.25 12.5 25 50 100 200 400 800
Wild type
akhr EY11371
Bitter sensitivity (sugar normalized PER)
E1
Wild type
E2
akhrEY11371 mutant
Fraction of flies not showing PER
All pairs n.s.
Fed
1 day WS
Bitter concentration (mM)
(mixed into normalized sugar sol.)
0 0.01 0.02 0.04 0.08 0.16 0.31 0.63 1.25 2.5


Bitter sensitivity (genetic control of Figure 6E)
F1
+/ +
F2
+ / UAS-dTrpA1
Fraction of flies not showing PER
All pairs n.s.
30 °C
18 °C
Bitter concentration (mM)
(mixed into 800 mM sucrose)
0 0.16 0.31 0.63 1.25 2.5 5 10


Sugar sensitivity
G1
+ / +
G2
+ / UAS-dTrpA1
G3
akh-GAL4 / +
G4
akh-GAL4 / UAS-dTrpA1
G5
sNPFc00448 mutant; akh-GAL4/ +
G6
sNPFc00448 mutant; akh-GAL4/ UAS-dTrpA1
Fraction of flies showing PER
All pairs n.s.
30 °C
18 °C
Sugar concentration (mM)
6.25 12.5 25 50 100 200 400 800

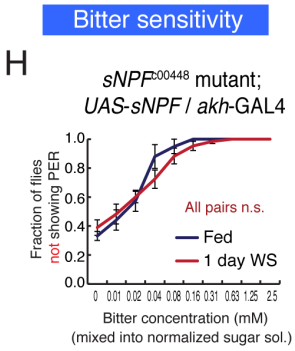

Bitter sensitivity
H
sNPFc00448 mutant;
UAS-sNPF / akh-GAL4
Fraction of flies not showing PER
All pairs n.s.
Fed
1 day WS
Bitter concentration (mM)
(mixed into normalized sugar sol.)
0 0.01 0.02 0.04 0.08 0.16 0.31 0.63 1.25 2.5

Figure S6

Figure S6. Genetic Manipulations of AKH Do Not Affect Sugar Sensitivity

(A-B) Sugar sensitivity of flies with genetic cell-ablation of AKH neuroendocrine cells. Data is acquired from the same flies that were used in Figure $6A_{1-3}$. S_{50} is summarized in Figure $6B_1$. Cell ablation was confirmed with loss of GFP signal (B_{1-2}). (C) Relative *akhr* mRNA expression level in *akhr*^{EY11371} flies compared to wild type flies in the same genetic background measured by qPCR. *P*-value represents t-test (n=3). (D) Sugar sensitivity of wild type and *akhr*^{EY11371} mutant flies in the same genetic background. Data is acquired from the same flies that were used in Figure $6C_{1-3}$. S_{50} is summarized in Figure $6D_1$. (E) Results of the sugar-normalized PER assay comparing bitter sensitivity between fed and 1-day WS wild type flies (E_1) and *akhr*^{EY11371} mutant flies (E_2) in the same genetic background were tested. Lobeline was mixed into 800 mM sucrose solution for fed flies, and 200 mM sucrose solution for 1-day WS flies. E_1 is the same as Figure $3C_1$ (duplicated for purposes of comparison). (F-G) Sugar and bitter sensitivity of flies with genetic thermoactivation of AKH-producing cells (w-; +; *akh-GAL4* (III) crossed with w-; +; + (G_3) or w-; *UAS-dTrpA1* (II); *UAS-dTrpA1* (III) (G_4). w-; *sNPF*^{c00448}; *akh-GAL4* (III) crossed with w-; +; + (G_5) or w-; *UAS-dTrpA1* (II); *UAS-dTrpA1* (III) (G_6)) and its genetic control flies (Wild flies crossed with w-; *UAS-dTrpA1* (II); *UAS-dTrpA1* (III) (F_2, G_2) or wild type flies in the same genetic background (F_1, G_1)). (H) Bitter sensitivity measured with the normalized-sugar PER assay in *sNPF* mutant flies with genetic rescue of sNPF expression in AKH neuroendocrine cells (w-; *sNPF*^{c00448}; *UAS-sNPF* crossed with w-; *sNPF*^{c00448}; *akh-GAL4*). Note that rescuing of *sNPF* expression in AKH neuroendocrine cells does not rescue the starvation-dependent decrease in bitter sensitivity. See also Figure $3C_{2-3}$ for comparison and sugar concentration used for the experiment. (A, D-H) n>4 for all experimental groups.

Supplemental Experimental Procedures

Fly strains

sNPF-GAL4, *UAS-sNPF*, and *UAS-sNPFR* (Lee et al., 2008) were generously provided by Drs. Kweon Yu and Jing W. Wang. *dnpf*-GAL4 (Wu et al., 2003), *akh-GAL4*(Lee and Park, 2004), *InsP3-GAL4* (Buch et al., 2008), *Gr66a-GAL4* (Scott et al., 2001), *Gr33a-GAL4* (Moon et al., 2009), *elav-GenesSwitch* (Osterwalder et al., 2001) were provided by Drs. Ping Shen, Jae H. Park, Michael J. Pankratz, Kristin Scott, Craig Montell, and Haig Keshishian, respectively. *BDP-GAL4* (a *GAL4* line with a *Drosophila* synthetic core promoter but no enhancer 5' to this promoter, which has been shown to have no detectable expression in the adult CNS (Pfeiffer et al., 2008)), and *n-synaptobrevin-GAL4* (*nsyb-GAL4*) (Pauli et al., 2008) were obtained from, Barret Pfeiffer, Drs. Gerald M. Rubin, and Julie Simpson, *UAS-mCD8::GFP* (pJFRC2 described in (Pfeiffer et al., 2010)), *UAS-GCaMP3.0* (Tian et al., 2009), *UAS-dTRPA1* (Hamada et al., 2008) were generously provided by Drs. Gerald M. Rubin, Dr. Loren L Looger, and Dr. Paul A. Garrity, respectively. RNAi and related lines (Dietzl et al., 2007) were generously provided by Dr. Barry J. Dickson via the VDRC stock center (*UAS-sNPFR RNAi* (GD661 v9379), *UAS-mCD8::GFP*, and *UAS-Dicer2* (on X chromosome)). $sNPF^{c00448}$, $sNPF^{f07577}$, and $akhr^{EY11371}$ were obtained from the Bloomington stock center and backcrossed for at least six generation into our wild type *Berlin* background. GMR GAL4 lines (Jenett et al., 2012) and tub-$Gal80^{ts}$ were also obtained from the Bloomington stock center. Other lines used in this research: *UAS-eGFP-KIR2.1*(Baines et al., 2001), *UAS-TeTxLC.TNT* (UAS-TNT), *UAS-TeTxLC.IMPTNT* (UAS-IMPTNT) (Sweeney et al., 1995).

All the wild type genetic background tested in this paper (Berlin, and Canton-S from Tully lab, Heisenberg lab, Janelia Farm, and VDRC) showed statistically significant starvation-dependent increase

in sugar sensitivity and decrease in bitter sensitivity. The baseline sugar and bitter sensitivity, and the amplitudes of starvation-dependent changes, however, vary among the backgrounds. Therefore, we performed statistical comparison only among the flies in the same genetic background. In addition, we performed experiments of all the control and experimental flies side by side on the same time of the day, so that circadian cycle and/or other slight differences in the environment do not affect the results. Since backcrossing was performed using *w-* flies, *w-* flies were used as "wild type" flies in this paper. Here, we listed the genetic backgrounds used for the experiments in this paper.

Wild type *Berlin* flies and flies backcrossed into this background	Figure 1B-E, 2A, B, E, H, 3A-E, 4F, 6C-F Figure S1C, S3A-D, S6C-H
Heterozygotes of Canton-S (from Tully lab) background flies and Canton-S (from Heisenberg lab) background flies	Figure 2F, G, 5A-B, 6A-B Figure S2B-C, S5A-B, S6A
Heterozygotes of Canton-S (from Janelia Farm) background flies and Canton-S (from VDRC) background flies	Figure 4A, E
Heterozygotes of Canton-S (from Tully lab) background flies and Canton-S (fromVDRC) background flies	Figure 2C-D, I Figure S2A, D-E
Heterozygotes of Canton-S (from Heisenberg lab) background flies and Canton-S (fromVDRC) background flies	Figure 5C-D, S5C-D,

PER assay and drug feeding

10-20 experimental flies were mounted into pipetman tips. After excluding flies that continually responded to water, fly response to stepwise increasing concentration of sucrose was tested. After testing the sugar sensitivity, the same sets of flies were tested for bitter sensitivity by exposing stepwise increasing concentration of lobeline mixed into 800mM or other concentrations of sucrose. Only full extensions, but not partial extensions, of proboscis were counted. We withdrew the drop as soon as possible after touching it to the labellum, so that flies could not drink the sucrose solution. Different concentration series of sucrose and lobeline were used depending on the genetic background so that the responses are within the dynamic ranges. All the control experiments were performed side by side as blind experiments. Description of sigmoid curve fitting is in the Supplemental Experimental Procedures.

For wet starvation (WS), flies were kept in a vial with a filter paper soaked with 1 ml of water. The filter paper was changed everyday in case of 2 days WS. For L-dopa feeding experiment, L-dopa precursor (Sigma-Aldrich) was dissolved in 89 mM sucrose solution. A filter paper was soaked with this L-dopa sucrose solution or 89mM sucrose solution (for control) to feed the flies for 2 days before experiments. For RU486 feeding, 10mM RU486 (Sigma-Aldrich) was dissolved in DMSO as a ×20 stock. Flies were moved into a vial with a filter paper soaked with 50 μl of RU486 stock solution or DMSO (for the vehicle fed condition) mixed into 1 ml of 89 mM sucrose solution and fed for 2 days. We confirmed that this concentration of DMSO does not affect sugar or bitter sensitivity. After this 2-day feeding, flies were wet starved for 1 day to test sugar/bitter sensitivity.

Immunohistochemistry

Dissected brains were fixed in 4% formaldehyde in PEM (0.1M PIPES, pH 6.95, 2mM EGTA, 1mM

MgSO4) for 2 hours at 4 °C. After three 15-min rinses with PBS, brains were incubated with primary antibodies overnight. Following three 15-min rinses with PBS, brains were incubated with secondary antibody overnight. Following three rinses, brains were incubated in 50% glycerol in PBS for 2 hours and cleared with VECTASHIELD® (VECTA). All procedures were performed in 4 °C. A Fluoview™ FV1000 Confocal laser scanning biological microscope (Olympus) with a 30×, 1.05 N.A. silicone oil objective (Olympus) was used to obtain confocal serial optical sections. For observation of native fluorescence, incubation with primary and secondary antibodies was omitted. The antibodies used: Rabbit Anti-sNPF precursor (Nassel et al., 2008) (kind gift by Dr. Dick R Nässel), Rabbit Anti-NPF (RB-19-001: RayBiotech), Mouse Tyrosine Hydroxylase Antibody (ImmunoStar), Alexa Fluor® 568 donkey anti Rabbit IgG(H+L), Alexa Fluor® 568 donkey anti Mouse IgG(H+L) (Invitrogen). Native GFP signal was observed without immunostaining.

ImageJ (NIH) was used to extract the voxels with overlap of GFP (green color) and anti-sNPF (magenta color) signal. These voxels were overlaid with white color to emphasize the overlap in Fihgure 4B and S4C. Fluorender software (Wan et al., 2009) was used to make 3D reconstructed images.

Sigmoidal fitting of data and statistics

In order to fit the data into a sigmoidal curve, sigmoid interpolation was performed. The sigmoid curves were defined as follows:

$$F_S = \frac{1}{1 + e^{(-\alpha_s \log_2 \frac{S_{con}}{S_{50}})}}$$

Where

F_S *: Fraction of flies showing the PER*

S_{con} : Concentration of sucrose

S_{50}: Sucrose concentration where 50% of flies show the PER

α_S : slope of the sigmoid curve

$$F_B = (1 - R_S) + \frac{R_S}{1 + e^{(-\alpha_B \log_2 \frac{B_{con}}{B_{50}})}}$$

Where

F_B : Fraction of flies not showing the PER

R_S : Fraction of flies showing PER when bitter is not mixed (The max PER ratio)

B_{con} : Concentration of lobeline

B_{50}: Bitter concentration required to inhibit the PER in 50% of flies that showed PER to sugar (without bitter)

α_B : slope of the sigmoid curve

Based on the experimentally measured quantities (S_{con} *or* B_{con} and $F_{S \text{ or } B}$), S_{50} or B_{50} and $\alpha_{S \text{ or } B}$ were chosen to best fit the data. For all experimental data, fitting based on nonlinear regression was calculated with Matlab (MathWorks). Goodness-of-fit was tested by two-way ANOVA between the sigmoidal curve and the actual PER response curve, which indicated a good fit for all cases ($p<0.05$. two-way ANOVA) (See supplementary Fig S1B for examples of fitting). Since we were interested in sugar and bitter sensitivity, we used S_{50} or B_{50} for data analysis.

The distributions of values of S_{50} and B_{50} were not significantly distinct from normal distribution among the data acquired from wild-type flies (null hypothesis that distribution is normally distributed was not

rejected by Lilliefors test: p=0.5 for fed flies, n=29, and p=0.29 for 1-day WS flies, n=17). Thus parametric tests were used for data analysis.

Calcium imaging

Two-photon imaging was performed on an Ultima two-photon laser-scanning microscope (Prairie Technology) with an imaging wavelength at 940nm. The protocol for calcium imaging was modified from that described in (Inagaki et al., 2012; Marella et al., 2006). After a brief anesthesia on ice, flies were mounted on a thin plastic plate with wax as shown in Figure 5E. The top side of the plate contained a well made with wax, and the fly head was immersed in ice-cold Ca^{2+} free saline (108mM NaCl, 5mM KCl, 8.2mM $MgCl_2$, 4mM $NaHCO_3$, 1mM NaH_2PO_4, 15mM Ribose, 5mM HEPES, pH 7.5; note that Ribose, which does not stimulate *Drosophila* sugar-sensing GRNs, is used instead of other sugars). In this saline bath, the antennae and cuticle at the anterior side of the fly head capsule were surgically removed with sharp forceps, so that the SEZ could be imaged. The fat body, air sacs, and esophagus were gently removed to give a clear view of the brain and to minimize its movement. At the bottom side of the plate, a glass tube was mounted with the opening facing the proboscis of the mounted fly. A piece of twisted Kimwipe was placed just behind the fly. During imaging, a lobeline solution was delivered from the glass tubing to stimulate gustatory neurons in the proboscis and was removed by the Kimwipe.

Following dissection, the ice-cold Ca^{2+} free saline was removed and the fly brain was immersed in 1 ml of room-temperature imaging saline (108mM NaCl, 5mM KCl, 2mM $CaCl_2$, 8.2mM $MgCl_2$, 4mM $NaHCO_3$, 1mM NaH_2PO_4, 15mM Ribose, 5mM HEPES, pH 7.5). This setup was moved under an Ultima two-photon laser scanning microscope (Prarie Instruments, Inc) with a 40× 0.8 N.A. objective (Olympus, Inc). The glass tubing was connected to four silicon tubes with a plastic manifold (MP-4, Warner Instruments).

Each silicon tube was connected to 50 ml syringes filled with either 15 ml of 0, 0.07, 0.31 or 1.25mM lobeline dissolved in water. The flow of lobeline solution was controlled using electrically triggered pinch valves (ALA-VM8, ALA Scientific Instruments) that compressed the silicon tubes between the syringes and the manifold. The timing of valve opening was controlled by the two-photon acquisition system and its software (Prairie view and Trigger Sync, Prairie) so that the timing was linked with the image acquisition. ΔF/F and peak ΔF/F were calculated using Matlab (MathWorks).

qPCR

RNA was extracted from heads of 10 flies (for *sNPF*) and whole bodies of 4 flies (for *akhr*). cDNA was synthesized using Super Script® VILO™ cDNA Synthesis kit (Invitrogen). Real Time PCR was performed using EXPRESS SYBR® GreenER™ (Invitrogen) and a 7300 Real Time PCR system (Applied biosystems). *Rp49* was used as a standard. Using melting temperature analysis, each primer pair was confirmed to produce a single PCR product. Primers listed below were used.

RP49-f: CCCGAAAACTTTTAGACTCA

RP49-r: TTTTCAAACATTTCCATCGT

sNPF-f: AGGGTATCGACAACAGAGTG

sNPF-r: CACCAGGAACTTCTTGAATC

AKHR-f: ACAACAATCCGTCGGTGAAC

AKHR-r: CTTCCATTCAGCAGCGAGTT

Supplemental References

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